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Effects of Repeated Antigen Exposure on Endothelin-1–Induced Bronchial Smooth Muscle Contraction and Activation of RhoA in Sensitized Rats

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Changes in endothelin-1 (ET-1)–induced contraction and activation of RhoA in bronchial smooth muscle of repeatedly antigen-challenged rats, which exhibit marked airway hyperresponsiveness (AHR), were examined. The ET-1–induced contraction of bronchial smooth muscle was significantly enhanced in the repeatedly antigen-challenged group. In normal control animals, ET-1 induced time- and concentration-dependent translocation of RhoA to the plasma membrane, indicating activation of RhoA by ET-1 in rat bronchial smooth muscle. The level of ET-1–induced RhoA translocation was increased much more markedly in the AHR group than in the control animals. It is suggested that the augmented activation of RhoA observed in the hyperresponsive bronchial smooth muscle might be responsible for the enhanced ET-1–induced contraction of bronchial smooth muscle in AHR rats.

Key words: airway hyperresponsiveness, asthma, bronchial smooth muscle, endothelin-1, Ca^{2+} sensitization, RhoA.

Abbreviations: ET-1, endothelin-1; AHR, airway hyperresponsiveness; DNP-Asc, 2,4-dinitrophenylated *Ascaris suum* extract; TTBS, Tris-buffered saline containing 0.1% Tween 20.

Smooth muscle contraction is induced by an increase in cytosolic Ca^{2+} *via* activation of plasma membrane Ca^{2+} channels and/or Ca^{2+} release from the sarcoplasmic reticulum. Additional mechanisms have also been suggested in agonist-induced smooth muscle contraction by studies involving simultaneous measurement of force development and the intracellular Ca^{2+} concentration (*1*), and chemically permeabilized preparations (*2*) in various type of smooth muscles including airway ones (*3*, *4*). It has been demonstrated that agonist stimulation increases myofilament Ca^{2+} sensitivity in β-escin–permeabilized smooth muscles of the rat coronary artery (*5*), guinea pig vas deferens (*3*), canine trachea (*6*), and rat bronchus (*4*). Although the detailed mechanism is not fully understood, the participation of RhoA protein, a monomeric GTP-binding protein, in agonist-induced Ca^{2+} sensitization has been suggested (*3*, *4*, *7*, *8*).

Bronchial asthma is an airway inflammatory disease characterized by increased airway responsiveness. Most asthmatic patients exhibit increased contractility of airway smooth muscles (*9*), which might be a major cause of airway hyperresponsiveness (AHR). Similarly, increased responsiveness of bronchial smooth muscle has also been demonstrated in a rat model of AHR induced by repeated antigen inhalation (*10–12*). In this animal model of AHR, the bronchial smooth muscle contraction induced by receptor agonists such as acetylcholine (ACh), but not by high K^+ depolarization, is markedly augmented (*10–12*). Moreover, it has also been found that the muscarinic receptor density and antagonist affinity of the airway smooth muscle are normal (*11*). Thus, it is possible that the mechanisms responsible for AHR exist, at least in part, in the downstream pathway of receptor signaling, including the agonist-mediated Ca^{2+} sensitization of smooth muscle contraction. Indeed, using β-escin–permeabilized bronchial smooth muscle, we recently revealed the occurrence of ACh-induced, RhoA-mediated Ca^{2+} sensitization in rat bronchial smooth muscle contraction and marked augmentation of this Ca^{2+} sensitizing effect in the increased smooth muscle contractility observed in AHR (*4*).

Endothelin-1 (ET-1) is a potent bronchoconstrictor (*13*), and an elevated level of ET-1 in the bronchoalveolar lavage fluids from asthmatic patients has been reported (*14*). In addition to the classical Ca^{2+}-mediated contraction (*15*), ET-1 also induces Ca^{2+} sensitization of contraction through activation of the RhoA/Rho-kinase pathway (*16*). In the present study, to determine whether or not the augmented Ca^{2+} sensitization of bronchial smooth muscle in AHR is mediated by enhancement of activation of RhoA, the ET-1–induced translocation of RhoA to the plasma membrane was investigated in bronchial smooth muscles of AHR rats. Although the activation pathway of RhoA *via* membrane receptors in smooth muscles is not yet clear, it is known that translocation of RhoA to the plasma membrane from the cytosol occurs when RhoA is activated (*3*, *8*, *17*).

MATERIALS AND METHODS

Sensitization and Antigenic Challenge—Male Wistar rats (6 weeks of age, specific pathogen–free, 170–190 g; Charles River Japan, Inc.) were sensitized and repeatedly challenged with 2,4-dinitrophenylated *Ascaris suum*

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antigen (DNP-Asc) by the method described in the previous papers (*4*, *10–12*). Age-matched non-sensitized normal animals were used as controls (*4*, *11*, *12*). All experiments were approved by the Animal Care Committee of Hoshi University (Tokyo, Japan).

Functional Study—About 4 mm length of the left main bronchus was isolated. Isometrical contraction of the circular smooth muscle was measured as described previously (*11*, *12*). ET-1 responsiveness was measured in the presence of atropine and indomethacin (both 10^{-6} M). In some experiments, the effect of Y-27632, an inhibitor of Rho-kinase, on ET-1 (10^{-6} M)–precontracted smooth muscle was also determined.

Preparation of Membrane and Cytosolic Fractions—Bronchial tissue preparations were prepared by the method described previously (*4*, *18*). In brief, the airway tissues below the main bronchi to the lungs were removed and immediately soaked in ice-cold, oxygenated Krebs-Henseleit solution (118.0 mM NaCl, 4.7 mM KCl, 2.5 mM $CaCl_2$, 1.2 mM $MgSO_4$, 25.0 mM $NaHCO_3$, 1.2 mM KH_2PO_4, 10.0 mM glucose, pH 7.4). The airways were carefully cleaned of adhering connective tissue, blood vessels and lung parenchyma under a stereomicroscope. As much as possible of the epithelium was removed by gently rubbing with keen-edged tweezers (*4*, *18*). Then the bronchial tissue (containing main and intrapulmonary bronchi) segments were equilibrated in oxygenated Krebs-Henseleit solution (37°C) for 60 min with 10-min washout intervals. After the equilibration period, the tissue segments were stimulated with a certain concentration of ET-1 for a certain time in the presence of atropine and indomethacin (both 10^{-6} M). The reaction was stopped by quickly freezing in liquid nitrogen and then the tissue was homogenized with Physcotron (Niti-on, Co., Ltd., Japan; 5 s × 6, max level) in 2-ml of ice-cold homogenization buffer of the following composition; 10 mM Tris-HCl (pH 7.5), 5 mM $MgCl_2$, 2 mM EDTA, 250 mM sucrose, 1 mM dithiothreitol, 1 mM 4-(2-aminoethyl) benzenesulfonyl fluoride, 20 μg/ml leupeptin and 20 μg/ml aprotinin. The tissue homogenate was centrifuged (105,000 × *g*, 4°C for 30 min) and the supernatant was collected as the cytosolic fraction. The pellet was resuspended in 3 ml of the homogenization buffer and recentrifuged (105,000 × *g*, 4°C for 30 min). The resultant pellet was resuspended in 2 ml of ice-cold homogenization buffer containing 1% Triton X-100 and 1% sodium cholate, and used as the membrane fraction. The preparations were stored at –80°C until use. The protein concentrations of these preparations were determined by the method of Lowry *et al*. (*19*) in triplicate with bovine serum albumin as a standard.

Western Blots—Immunoblotting was performed using the preparations obtained from the AHR (that were sensitized and repeatedly antigen-challenged) and normal control rats as described above. Briefly, the membrane and cytosolic fractions were dissolved in SDS sample buffer and then heated at 100°C for 4 min. Samples (10 μg protein per lane) were subjected to 15% SDS-PAGE. Proteins were then electrophoretically transferred for 4 h onto nitrocellulose membranes (Hybond-ECL, Amersham, UK) in cold transfer buffer (20% methanol containing 25 mM Tris and 192 mM glycine). After repeated washing with Tris buffer (20 mM Tris, 500 mM NaCl, pH 7.5) containing 0.1% Tween 20 (TTBS), the nitrocellulose membranes were incubated with blocking buffer (3% gelatin in TTBS) for 1.5 h at room temperature. The nitrocellulose membranes were then incubated with polyclonal rabbit anti-RhoA (amino acids 119–132, 1:3,000 dilution; Santa Cruz) in antibody buffer (1% gelatin in TTBS) for 12 h at room temperature. The nitrocellulose membranes were then washed 5 times (for 15 min each) with TTBS. They were then incubated with horseradish peroxidase (HRP)–conjugated goat anti-rabbit IgG (1:5,000 dilution; Amersham) for 1.5 h at room temperature, and washed 5 times with TTBS. The blots were examined by an enhanced chemiluminescent method (ECL System; Amersham) and quantitated with a densitometry system (Atto Densitograph Software ver.4.0; Atto Co., Japan). To normalize the RhoA contents by an internal control protein, β-actin, immunoblotting was performed on the same gel with monoclonal mouse anti–β-actin N-terminal (1:5,000 dilution; Sigma, St. Louis, MO) and goat anti-mouse IgG (1:5,000 dilution; Amersham). Under the above conditions, a linear relationship between the band density of RhoA and the amounts of loaded proteins was found for protein concentrations ranging from 5 to 25 μg (*4*). Similar result was obtained for β-actin. The ratios of corresponding RhoA/β-actin in each lane were calculated as an index of the RhoA protein level. The percentage of membrane/total RhoA was calculated according to (membrane RhoA/β-actin)/[(membrane Rho/β-actin) + (cytosolic RhoA/β-actin)].

RhoA Pull-Down Assay—The active form of RhoA, GTP-bound RhoA, in BSMs was measured by means of the RhoA pull-down assay as previously described (*20*). In brief, bronchial tissues containing the main and intrapulmonary bronchi were isolated as described above. The isolated bronchial tissues were equilibrated in oxygenated Krebs-Henseleit solution at 37°C for 1 h. After the equilibration period, the tissues were stimulated with ET-1 (10^{-6} M for 10 min) and then quickly frozen with liquid nitrogen. The tissues were then lysed in lysis buffer of the following composition (mM); HEPES 25.0 (pH 7.5), NaCl 150, IGEPAL CA-630 1%, $MgCl_2$ 10.0, EDTA 1.0, glycerol 10%, NaF 25.0, sodium orthovanadate 1.0 and peptidase inhibitors. Active RhoA in tissue lysates (200 μg protein) was precipitated with 25 μg GST-tagged Rho-binding domain (amino acids residues 7–89 of mouse rhotekin; Upstate, Lake Placid, NY, USA), which was expressed in *Escherichia coli* and bound to glutathione-agarose beads. The precipitates were washed three times in lysis buffer, and then after adding the SDS loading buffer and boiling for 5 min, the bound proteins were resolved in 15% polyacrylamide gels, transferred to nitrocellulose membranes, and then immunoblotted with anti-RhoA antibodies as described above.

Statistical Analyses—All the data were expressed as means with SEM. The statistical significance of differences was determined by means of Dunnett's multiple analysis or two-way analysis of variance (ANOVA). A value of $p < 0.05$ was considered significant.

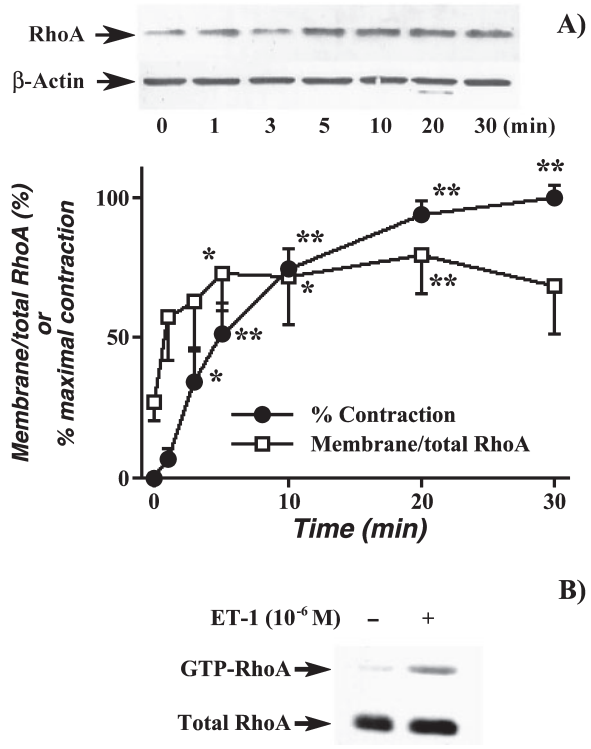

Fig. 1. **Relevance of the time courses of endothelin-1 (ET-1; 1 μM)–induced contraction and RhoA translocation.** A: The ratios of membrane to total RhoA protein (Membrane/Total RhoA) were calculated as described under "MATERIALS AND METHODS." The values are means with SEM for 4–6 experiments performed in duplicate. $*p < 0.05$ and $**p < 0.01$ *vs.* respective time 0 basal levels (no stimulation). Upper panel: Representative Western blots of membrane RhoA (21 kDa) and β-actin. B: Activation of RhoA by ET-1. Isolated bronchial tissues were incubated for 10 min in the absence (–) or presence (+) of 1 μM ET-1. The tissues were then rapidly lysed, the GTP-bound active form of RhoA was pulled down with the GST-tagged Rho binding domain of rhotekin, and RhoA was visualized by Western blotting. The respective blot of total RhoA in each sample is also shown. The data shown are representative of 3 independent experiments.

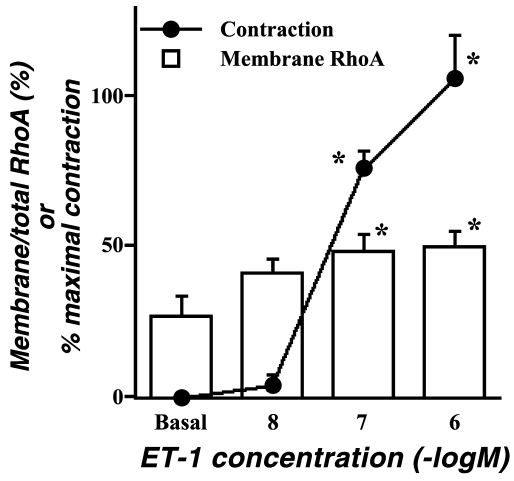

Fig. 2. **The concentration-dependency of endothelin-1 (ET-1)–induced translocation of RhoA and contraction in bronchial smooth muscle of nonsensitized normal rats.** Isolated main and intrapulmonary bronchi were stimulated with ET-1 (10^{-8}–10^{-6} M), and then homogenized to prepare cytosolic and membrane fractions after stopping the reaction with liquid nitrogen 20 min after stimulation. Western blotting was performed using these fractions for both RhoA and β-actin in an identical membrane. The values are means with SEM for 4–6 experiments performed in duplicate. $*p < 0.05$ *vs.* respective basal level (no stimulation).

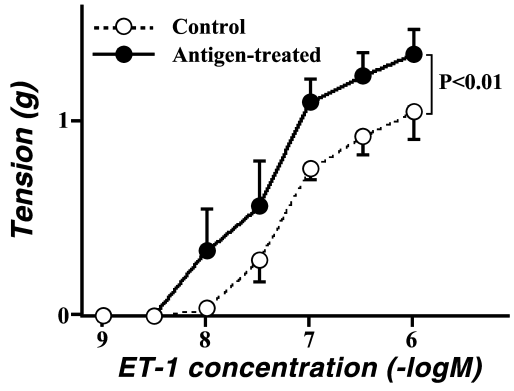

Fig. 3. **Endothelin-1 (ET-1) concentration-response curves for contractile responses of isolated bronchial rings from nonsensitized normal (Control) and repeatedly antigen-challenged rats (Antigen-treated).** Each value is the mean with SEM for 8 (Control) or 6 (Antigen-treated) experiments. The ET-1 responsiveness was significantly augmented in the antigen-treated group ($p < 0.01$ by ANOVA).

RESULTS

Contraction and RhoA Translocation Elicited by ET-1 in Bronchial Smooth Muscle of Control Rats—In normal control rats, both the membrane and cytosolic fractions of bronchial smooth muscle contained RhoA proteins (RhoA/β-actin, $n = 5$: 0.12 ± 0.04 and 0.42 ± 0.18, respectively) in the resting state (no ET-1 stimulation). The ratio of membrane to total RhoA in the resting state was 27.2 ± 6.8%. ET-1 (10^{-6} M) stimulation elicited increases in tension and membrane RhoA (Fig. 1A), while cytosolic RhoA was decreased (not shown), *i.e.*, translocation of RhoA to the plasma membrane. One minute after the addition of ET-1 (10^{-6} M) to the bronchial smooth muscle, membrane RhoA (membrane/total RhoA) rapidly increased to 57.3 ± 15.5% ($n = 5$), this change being followed by an increase in tension. Thereafter, both membrane RhoA and contraction gradually increased, and reached plateaus at 5–10 and 20–30 min after ET-1 stimulation, respectively (Fig. 1A). As shown in Fig. 1B, the GTP-bound active form of RhoA was also distinctly detected in the ET-1 (1 μM, for 10 min)–stimulated muscle strips. ET-1 also induced concentration-dependent increases in tension and membrane RhoA (Fig. 2). The ET-1 (10^{-6} M)–induced increase in membrane RhoA was completely blocked by treatment with an ET_A receptor antagonist, BQ-123, whereas an ET_B receptor antagonist, BQ-788, had no significant effect (preliminary study).

Effects of Repeated Antigen Exposure on ET-1–Induced Contraction and RhoA Translocation—The ET-1 responsiveness of the bronchial smooth muscles isolated from normal control and repeatedly antigen-challenged rats is shown in Fig. 3. ET-1 elicited a concentration-dependent contractile response in both groups. The concentration-

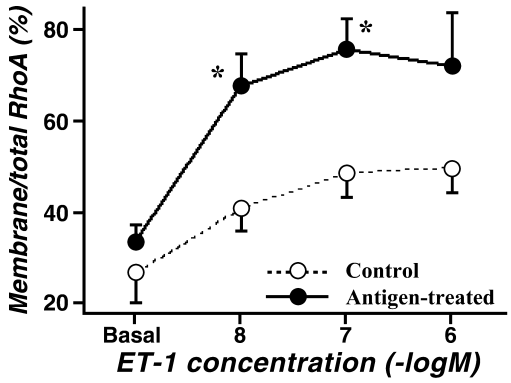

Fig. 4. **Endothelin-1 (ET-1) concentration-response curves for the increase in membrane RhoA, that is, RhoA translocation to the membrane, in bronchial smooth muscles from nonsensitized normal (Control) and repeatedly antigen-challenged rats (Antigen-treated).** Isolated main and intrapulmonary bronchi were stimulated with ET-1 (10^{-8}–10^{-6} M), and then homogenized to prepare cytosolic and membrane fractions after stopping the reaction with liquid nitrogen 20 min after stimulation. Western blotting was performed using these fractions for both RhoA and β-actin in an identical membrane. The values are means with SEM for 4–6 experiments performed in duplicate. *$p < 0.05$ *vs.* Control.

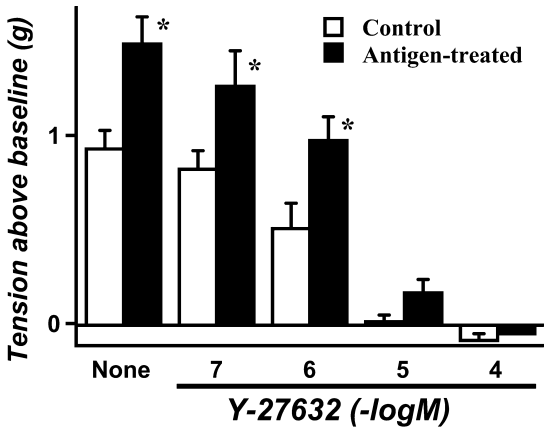

Fig. 5. **Effects of a Rho-kinase inhibitor, Y-27632, on endothelin-1 (ET-1, 10^{-6} M)–precontracted bronchial smooth muscles of nonsensitized normal (Control) and repeatedly antigen-challenged rats (Antigen-treated).** After the plateau contraction induced by ET-1 (10^{-6} M) was observed (None), Y-27632 (10^{-7}–10^{-4} M) was administered cumulatively. The values are means with SEM for 5 experiments, respectively. *$p < 0.05$ *vs.* Control. In both groups, the ET-1–induced contraction was reversed completely by Y-27632, indicating a major role of the RhoA/Rho-kinase pathway in the ET-1–induced bronchial smooth muscle contraction.

response curve for ET-1 was significantly shifted upward in the repeatedly antigen-challenged group. In the bronchial smooth muscle of the repeatedly antigen-challenged rats, the basal RhoA contents (no ET-1 stimulation) estimated with RhoA/β-actin were significantly greater (0.61 ± 0.26 in membrane and 1.16 ± 0.17 in cytosolic fractions, $n = 5$) than those in normal animals (0.12 ± 0.04 and 0.42 ± 0.18, respectively; $p < 0.05$), whereas the ratio of membrane to total RhoA remained within normal range (32.1 ± 7.3%; Fig. 4). As shown in Fig. 4, ET-1 elicited concentration-dependent translocation of RhoA in both groups. Interestingly, the ET-1 concentration–response curve for translocation of RhoA in the repeatedly antigen-challenged rats was significantly shifted upward as compared to that for normal animals ($p < 0.05$ by ANOVA; Fig. 4).

Effect of Y-27632 on ET-1–Precontracted Bronchial Smooth Muscle—In the bronchial smooth muscles of control rats that had been precontracted with 10^{-6} M ET-1, cumulatively administered Y-27632 (10^{-7}–10^{-4} M) induced concentration-dependent relaxation (Fig. 5). The ET-1–induced contraction was reversed completely by 10^{-5} M Y-27632, indicating a major role of the RhoA/Rho-kinase pathway in the ET-1–induced contraction. In the bronchial smooth muscles of the repeatedly antigen-challenged rats, the tension was still significantly greater than in the control group even in the presence of 10^{-7} and 10^{-6} M Y-27632, but was also reversed completely by a higher concentration of Y-27632 (Fig. 5).

DISCUSSION

Our previous study demonstrated that repeated challenge with an aerosolized antigen of actively sensitized rats (the same methods as those used in the present study) causes distinct airway inflammation and remarkable AHR to inhaled acetylcholine (ACh) *in vivo* (*10*). The isolated bronchial smooth muscles from these animals also exhibited hyperresponsiveness to ACh (*4*, *10–12*). Marked augmentation of ACh-induced, RhoA-mediated Ca^{2+} sensitization has also been demonstrated in bronchial smooth muscle in this animal model of AHR (*4*). Moreover, ACh-induced translocation of RhoA in AHR rats was significantly increased (*18*), indicating that ACh-mediated activation of RhoA in bronchial smooth muscle is augmented in antigen-induced AHR. Here, both the contraction and RhoA translocation induced by ET-1, a potent bronchoconstrictor that stimulates receptors other than muscarinic ones, were also augmented. Thus, the bronchial smooth muscle hyperresponsiveness might result from augmented intracellular signal transduction such as RhoA activation but not changes in plasma membrane receptors. Indeed, no change in the density of muscarinic receptors has been demonstrated in AHR (*11*).

Although the details are not fully known for airway smooth muscle, the mechanism of translocation of RhoA has been proposed to be as follows. In the resting state, the GDP-bound inactive form of RhoA exists in the cell cytosol with GDP dissociation inhibitor, called RhoGDI, which buries the geranylgeranylated, hydrophobic tail of RhoA. Activation of RhoA is initiated by guanine nucleotide exchange factors (RhoGEFs) through activation of plasma membrane receptors coupled to certain heterotrimeric G proteins. The active RhoGEFs exchange GDP for GTP on RhoA. Then RhoGDI dissociates from the GTP-bound active form of RhoA, resulting in the association of GTP-bound RhoA with the membrane (*21*). So in the present study, membrane-bound RhoA was measured by immunoblotting to determine the activation of RhoA by ET-1. In normal rats, both the membrane and cytosolic fractions of bronchial smooth muscle contain RhoA proteins (RhoA/β-actin, $n = 5$; 0.12 ± 0.04 and 0.42 ± 0.18, respectively) in the resting state (no ET-1 stimulation). The ratio of membrane to total RhoA in the resting state

(27.2 ± 6.8%) is approximately that reported for the portal vein and ileum (*22*), and in our previous paper (*18*), indicating successful separation of the membrane RhoA proteins. ET-1 (10^{-6} M) stimulation elicited increases in tension and RhoA content in the membrane fractions (Fig. 1), while cytosolic RhoA was decreased (not shown). One min following the addition of ET-1 (10^{-6} M) to bronchial smooth muscles, membrane RhoA rapidly increased by 57.3 ± 15.5% and then reached a plateau within 10 min. The ET-1–induced contraction was followed by the RhoA translocation and a plateau response was observed at 20–30 min after ET-1 stimulation (Fig. 1). The time-course change in the RhoA translocation of bronchial smooth muscle (Fig. 1) is similar to that of portal vein smooth muscle (*8*), which revealed a correlation between RhoA translocation and Ca^{2+} sensitization. However, the current time-course of RhoA translocation might be different from that of RhoA activation in human umbilical vein endothelial cells (*23*). There may be some difference in the activation mechanism for the RhoA protein (*e.g.*, smooth muscle *vs*. endothelium).

As shown in Fig. 2, ET-1 also induced concentration-dependent increases in tension and membrane RhoA. The ET-1 (10^{-6} M)–induced increase in membrane RhoA was completely blocked by treatment with an ET_A receptor antagonist, BQ-123, whereas an ET_B receptor antagonist, BQ-788, had no significant effect (preliminary study). On the other hand, depolarizing stimulation induced by isotonic 60 mM K^+ had no effect on the localization of RhoA (*18*), although distinct contraction was observed. These findings suggest that ET-1 induces translocation of RhoA from the cytosol to the membrane, *i.e*., activation of RhoA (*8*, *17*, *22*), in rat bronchial smooth muscles probably *via* activation of ET_A receptors. Although the reason for the time lag between ET-1–induced contraction and RhoA translocation (Fig. 1), which was not observed so clearly when ACh was used (*18*), is not known at present, some difference in signaling between heterotrimeric G proteins and RhoA may be involved. ET_A receptors activate both G_q and G_{13} proteins (*24*), whereas muscarinic M_3 receptors mainly activate G_q. Smooth muscle contraction is regulated by myosin light chain phosphorylation, which is intricately modulated by myosin light chain phosphatase, RhoA/Rho-kinase and CPI-17. It has been reported that ET-1 induces dephosphorylation of CPI-17, an endogenous myosin light chain phosphatase inhibitor, via activation of ET_B receptors (*24*). The inhibition of CPI-17 through its dephosphorylation may affect smooth muscle contraction induced by ET-1.

As shown in Fig. 3, ET-1–induced bronchial contraction was significantly augmented in the repeatedly antigen-challenged rats, indicating that reproducible and non-specific AHR occurs at the level of the bronchial smooth muscle after repeated antigen inhalation (*4*, *10–12*). In this animal model of AHR, the basal RhoA contents (no ET-1 stimulation) estimated as RhoA/β-actin were significantly greater (0.61 ± 0.26 in membrane and 1.16 ± 0.17 in cytosolic fractions, $n = 5$) than those in normal animals (0.12 ± 0.04 and 0.42 ± 0.18, respectively; $p < 0.05$), whereas the ratio of membrane to total RhoA was within normal range (32.1 ± 7.3%). The increased content of RhoA in bronchial smooth muscles from AHR rats is consistent with our previous report (*4*). Although the basal content of bronchial membrane RhoA was increased in AHR, the functional role of this membrane-associated RhoA is unclear. Gong *et al*. (*8*) suggested that membrane-associated RhoA exists in at least two states, *i.e*., resting and activated states, in the rabbit portal vein. With no ET-1 stimulation, a large portion of the membrane-associated RhoA observed in the hyperresponsive bronchial smooth muscle may be in the resting state.

As in the case of the normal group, ET-1 stimulation induced concentration-dependent translocation of RhoA to the membrane in bronchial smooth muscles from AHR rats (Fig. 4). The ET-1 concentration-response curve for translocation of RhoA in the AHR rats was significantly shifted upward as compared to that in normal animals ($p < 0.05$ by ANOVA; Fig. 4). This finding indicates that ET-1–induced translocation of RhoA is enhanced in the bronchial smooth muscles of AHR rats. Thus, in addition to the upregulation of RhoA proteins (*4*), it is suggested that the RhoA activation pathway(s) of bronchial smooth muscles might be functionally augmented in AHR in rats. This hypothesis may be also supported by the finding that the ET-1–induced contraction was reversed completely by Y-27632, a Rho-kinase inhibitor, in both groups (Fig. 5). Although the details of the mechanism of the activation pathway for RhoA in airway smooth muscle is not clear at present, the involvement of heterotrimeric G proteins has been suggested (*24–26*). We also demonstrated upregulation of G_q (*27*) and G_{13} (*28*) in bronchial smooth muscle in the AHR model. Therefore, it is possible that enhanced signaling by the increased heterotrimeric G proteins may be involved in the augmented activation of RhoA in AHR in rats.

In conclusion, ET-1 induced RhoA translocation to the plasma membrane, that is, activation of RhoA, in rat bronchial smooth muscle. The ET-1–induced RhoA translocation was markedly increased in the AHR rats as compared with in the control group. Our data suggest that the augmented activation of RhoA observed in the hyperresponsive bronchial smooth muscle might be responsible for the enhanced ET-1–induced bronchial smooth muscle contraction in AHR in rats.

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